a two-month extension of time, a response is now due December 22, 2009. Accordingly, this response is being timely filed.

Please amend the subject application as follows:

Amendments to the Specification:

Please amend the paragraph on page 1, lines 20 to 34 of the specification as follows:

> Vertebrate cells have evolved a number of defense mechanisms to prevent or inhibit viral replication after an infection. A remarkable array of such antiviral proteins are induced by interferon (2), including: <u>protein kinase R (PKR)</u> ~~PKR~~, a double-stranded RNA-dependent kinase that phosphorylates eIF-2α and shuts down translation (3); the <u>myxovirus-resistance (Mx)</u> ~~Mx~~ proteins, GTPas[s]es that block viral gene expression by unknown mechanisms[m] (4); and oligoA synthetases (5), producing 2', 5'-oligodenylates (6) that activate Rnase L to degrade both mRNAs. In some cases the antiviral state involves a drastic shutoff of host functions. In other cases, there is a more specific block to viral replication or gene expression. While many parallel pathways have been uncovered, it is likely that still more antiviral proteins remain to be found.

Please amend the paragraph on page 2, line 3 of the specification as follows:

This invention provides an isolated Zinc-finger antiviral protein (ZAP)~~ZAP~~ protein.

Please amend the paragraph on page 7, lines 4 to 14 of the specification as follows:

Figure 3B Blast search of Genbank database with NZAP fragment identified two mouse EST clones (mEST995 and mEST896) that have high sequence similarity to rZAP. These two clones were obtained from ATTC and sequences. The 3' end sequence of mEST995 was used to design a primer to PCR amplify full-length rZAP from Rat2 cDNA library. Deduced amino acid sequences are compared schematically. The numbers of amino acids of each coding sequence are indicated. The positions of four CCCH finger motifs are indicated by black boxes and the sequences of the motifs in rZAP are shown (SEQ ID NO:11-14).

Please amend the paragraph on page 8, lines 1 to 16 of the specification as follows:

Figure 4C Analysis of viral RNA levels in infected cells. Cells infected with undiluted Eco-Luc virus, and forty-eight hours post infection total RNA, nuclear RNA and cytoplasmic RNA fractions were isolated. 20 micrograms of RNA from each fraction was resolved by electrophoresis in agarose gel and then transferred to a Nylon membrane. The membrane was probed with P32-labeled luciferase DNA and exposed to X-ray film (top panel). The same membrane was stripped ~~stipped~~ and re-probed with 32P-labeled glyceraldehyde-3-phosphate dehydrogenase (GAPDH) ~~GAPDH~~ DNA [a]and exposed to X-ray film (middle panel).

To monitor the integrity of the RNA samples, the gel was stained with ethidium bromide (bottom panel) before transfer to the membrane. The relative RNA levels were quantitated by Phosphoimager and plotted. The positions of each RNA are indicated. E, Rat2-empty vector control cells; Z, Rat2-NZAP-zeo cells.

Please amend the paragraph starting on page 19, line 23 and ending on page 21, line 3 of the specification as follows:

A library of expressed cDNAs was constructed in a retroviral vector, termed pBabe-HAZ. This vector was constructed by making modifications to pBabe-puro. The EcoRI and NotI sites in pBabe-puro were sequentially removed by digestion, polishing of the ends by Klenow polymerase and ligation. The puromycin resistance gene was replaced by a zeocin resistance gene prepared by PCR with various components built in the primers. The upstream primer (5'ATAAGCTTGCCACCATGGCTTSTCCSTSTGSTGTTCCAGATATGCTGAATTCGGCGGCCGCGCCAAGTTGACCAGTGC-3') <u>(SEQ ID NO:3)</u> contained the HindIII cloning site, kozak consensus sequence, ATG start codon, HA tag and ECORI/NotI linker sequences, with HA tag fused to the zero gene. The downstream primer 5'ATATCGATTCAGTCCTGCTCCTCGGC-3') <u>(SEQ ID NO:4)</u> contained the ClaI cloning site. The Lox P sequence was inserted by annealing two oligonucleotides (5'CTAGATAACTTCGTATAATGTATGCTATACGAAGTTAT-3') <u>(SEQ ID NO:5)</u> and (5'CTAGATAACTTCGTATAGCATACATTATACGAAGTTAT-3') <u>(SEQ ID NO:6)</u> and ligating the product into the unique Nhel site in the U3 region of the 3' LTR. To minimize the background of

parental vector in the cDNA library, a 1-kb stuffer sequence was inserted between the EcoRI and NotI sites to disrupt the HA-Zeo open reading frame. cDNAs were then used to replace the stuffer. (Fig. 1A). Randomly primed cDNAs from wild-type Rat2 fibroblasts were inserted into the vector under the control of a constitutive promoter, such that a hemagglutinin (HA) epitope tag was fused at the 5' end, and a Zeocin resistance gene at the 3' end, encoding HA-orf-Zeo fusion proteins. RNA extracted from RAT2 cells with RNA extraction kit (Amersham-Pharmacia) following the manufacturer's instructions. cDNA was synthesized from the mRNA and cDNA synthesis kits (Stratagene) following the manufacturer's instructions with the following modifications: a) NotI-oligo(dT) primer (Amersham-Pharmacia) was used to replace XhoI-oligo(dt) with the NotI-oligo(dT) primer replace XhoI-oligo(dt); b) for each reaction, 15ug, instead of 5 mg of mRNA was used as template to favor the synthesis of short cDNA fragments. The cDNA was cloned into pBabe-Haz digested with EcoRI and NotI and the reaction products were used to transform Electromax bacteria by electroporation. A LoxP sequence, the site recognized by the Cre recombinase, was inserted into the U3 region of the 3' Long Terminal Repeat (LTR), which is duplicated during reverse transcription of the vector so that LoxP sites[d] are present in both LTRs of the provirus after integration. These sites are positioned such that the provirus can be excised from the genome by the Cre recombinase. The complexity of the library was 2 x 10^7, with inserts ranging in size from 0.2 kb to 3 kb.

Please amend the paragraph starting on page 22, line 33 and ending on page 23, line 17 of the specification as follows:

The cDNA insert in L1D3 was recovered from the genomic DNA by PCR amplification and cloned. To recover the cDNA insert from the L1D3 cell line, 1mg of genomic DNA was used as a template in a 50 ml PCR reaction with the Expand High Fidelity PCR kit under the following conditions: 10 cycles of 94oC for 15 seconds, 50oC for 30 seconds, 72oC for 60 seconds each cycle, followed by 20 cycles of 94oC for 15 seconds, 55oC for 30 seconds, 72oC for 60+5 seconds each cycle. The sense primer was 5'GCTTATCCATATGATGTTCCAGATT-3' (SEQ ID NO:7), and the antisense primer was CZAP-ap-AP (5'ATATAGGCGGCCGCCCTCTGGACCTCTTCTCTTC-3') (SEQ ID NO:8). To confirm that the cDNA was sufficient to induce virus resistance, the cDNA was recloned into the pBabe-HAZ vector and then reintroduced into naive Rat2 cells. Cells expressing the cDNA were again 30-fold resistant to the Eco-Luc virus as compared to the parental cells or cells carrying the empty vector (Fig. 2B). Thus, the expression of the cDNA was sufficient to establish viral resistance.

Please amend the paragraph starting on page 23, line 19 and ending on page 24, line 26 of the specification as follows:

The DNA sequence of the insert revealed a single long open reading frame of 254 codons fused to the zeocin resistance gene at its 3' end (Fig. 3A). The insert contained a long 5' untranslated region (UTR) and the ORF was not fused to HA at the 5' end; translation of the HA sequence terminated in the

UTR and expression of the protein required translation initiation at an AUG codon at the start of the ORF, in the context of a good match to a Kozak consensus start site. Searches of the nucleic acid databases with the coding region revealed two mouse EST clones with highly similar sequences (mEST995 and mEST896). These two sequences were identical to each other except for differences at their very 3' ends, which probably arise by alternative splicing events. The sequence of mEST995 was used to design PCR primers, and the full-length sequence of the rat cDNA was amplified and cloned. The C-terminal portion of ZAP was cloned from a Rat2 cell cDNA library by PCR using sense primer CZAP-SP (5'GAGCTCTCTGGGCTTAACC-3') (SEQ ID NO:9) and antisense primer CZAP-AP (5'ATATAGGCGGCCGCCCTCTGGACCTCTTCTCTTC-3') (SEQ ID NO:10). The sense primer lies upstream from an internal NheI site in NZAP; the antisense primer introduces a NotI site (bolded) immediately downstream from the coding sequence to facilitate its cloning into the myc-tagged expression vector. PCR was conducted with Expand High Fidelity PCR kit (Roche) under the following conditions: 10 cycles of 94oC for 15 seconds, 50oC for 30 seconds, 72oC for 120 seconds each cycle, followed by 20 cycles of 94oC for 15 seconds, 55oC for 30 seconds and 72oC for 120+5 seconds each cycle. The PCR product was digested with NheI and NotI and then cloned into pCDNA4/T02-NZAP-myc. The sequence of the complete cDNA contained 789 codons (sequence deposited in GenBank, accession # pending); the initial cDNA corresponded perfectly to the aminoterminal one-third of the sequence. The predicted amino acid sequences of the rat protein and of the similar mouse proteins contained a cluster of four unusual CCCH-type

zinc fingers, previously found in only a few RNA-binding proteins (Fig 3B). The gene was dubbed rZAP, for rat Zinc-finger Antiviral Protein, and the initial antiviral N-terminal fusion construct was named NZAP-zeo.

Please amend the paragraph on page 28, lines 10 to 29 of the specification as follows:

ZAP prevents the accumulation of cytoplasmic viral RNA because of the presence of the cluster of four unusual CCCH-type zinc fingers suggests that ZAP may interact directly with the viral RNA. These fingers are found in a small family of RNA binding proteins; the best-known member of the family is tristetraprolin (TTP), a protein which negatively regulates the levels of TNF-$\underline{\alpha}$ ~~ec (24)~~ (10) and GM-CSF mRNAs ~~(25)~~ (3). TTP binds AU-rich sequences in the 3' UTR of the TNF-$\underline{\alpha}$ ~~a~~ mRNA ~~(24,26)~~ (10,11) and recruits the exosome to degrade the mRNA ~~(27)~~ (4); it acts in opposition to the binding of HuR, another RNA-binding protein which stabilizes its target. rZAP may act in a similar way at sequences found in viral RNAs, and perhaps also in specific cellular mRNAs. Consistent with this notion, preliminary tests of ZAP mutants suggest that all of the finger motifs are crucial for its activity (data not shown). However, there is little sequence similarity to TTP outside the fingers, and the distinctive parts of the molecule may carry out other functions than the induction of RNA degradation.